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Aerobic Biodegradation of Strychnine Alkaloid Rodenticide in Soil

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ABSTRACT

An aerobic soil biodegradation study was conducted with strychnine alkaloid to evaluate loss of the parent compound (non-radiolabeled) from sandy loam and sandy clay loam soils during 2 months of incubation, and to detect non-volatile products which occurred during this period. The biologically active soil samples were treated with the strychnine alkaloid to yield an overall concentration of 10 ppm. The treated samples and controls were held in an environmental chamber under dark conditions at a temperature of 25°C and a soil moisture content of 75% of field capacity. Eight sampling periods were chosen; at each time interval, three treated samples and a control were selected for strychnine extraction and analysis using high performance liquid chromatography (HPLC/u.v.) at a wavelength of 254 nm.

Degradation of strychnine in both the sandy loam and sandy clay loam soils occurred in three distinct phases, which included a lag phase, a rapid loss phase and a leveling off or soil binding phase. It is believed that the lag phase may have been due to a microbial adaptation period combined with soil sorption. Approximately 50% of the strychnine was lost from the sandy loam and sandy clay loam soils in 24 and 27 days, respectively. Within a period of 33–40 days, about 90% of the strychnine had dissipated from both soils. The appearance of a degradation product occurred early in the study (day 7) and reached a maximum concentration at either day 14 (sandy loam) or day 21 (sandy clay loam). The initial degradates of strychnine are believed to be polar compounds with strong sorption characteristics. A discussion is presented in this paper of these possible products, together with a mechanism by which strychnine is theorized to have degraded in the soils.

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INTRODUCTION

Limited data are available regarding the fate of the plant alkaloid strychnine ($C_{21}H_{22}N_2O_2$), when used as a vertebrate damage control minor-use pesticide. Thus, in response to various environmental concerns and to comply with EPA reregistration data requirements, aerobic soil biodegradation research was initiated at our Center.

Few data are available concerning the degradation of strychnine in soil or aqueous systems. Howard *et al.* (1991) provided both low (1 week) and high (4 weeks) 'half-life' estimates for strychnine in aqueous media.

In addition, biotransformation data for strychnine are available in other biological systems, i.e. *in vitro* and *in vivo* metabolism in rats/rabbits and in isolated bacterial and fungal cultures. Results of these studies are briefly discussed below.

The *in vitro* metabolism of strychnine was studied in supernatant fractions from rabbit and rat liver tissues (Mishima *et al.*, 1985). During a 1-h incubation period, about 71% of the applied strychnine disappeared from the incubation mixture at an optimum pH of 8·4. The major metabolite identified was strychnine *N*-oxide, which accounted for about 15% of the metabolized strychnine (Fig. 1). The major route of biotransformation was catalyzed by cytochrome P-450 and/or FAD-containing monooxygenase. Another metabolic pathway may have involved the epoxidation of the double bond between C-21 and C-22, followed by hydrolysis to 21α, 22α-dihydroxy-22-hydrostrychnine. It was proposed that the 11,12-dehydrostrychnine metabolite (Fig. 1) was derived from an hydroxyl metabolite. Also, this research suggested the presence of a large number of metabolites which were neither isolated nor identified. An *in vivo* rat study was conducted by Oguri *et al.* (1989) in which urinary and fecal samples were collected and analyzed for radioactivity during a 7-day collection period; the rats had been dosed subcutaneously with ^{3}H-strychnine. Results of this study indicated that urinary and fecal excretions of radioactivity were about 30 and 65% of the dose in 7 days, respectively; however, most of the radioactivity was excreted within 1 day. Approximately 6 and 3% of the dose was excreted into urine and feces, respectively, as the parent strychnine. Most metabolites were more polar than strychnine, with radiochromatograms indicating the presence of metabolites corresponding to strychnine *N*-oxide, 21α,22α-dihydroxy-22-hydrostrychnine, 2-hydroxystrychnine and 16-hydroxystrychnine (Fig. 1). Considerable activity was reported to have remained in the aqueous layer after extraction with chloroform-isopropanol, which would indicate the presence of other polar metabolites. The major metabolite of strychnine was a stable product identified as strychnine 21,22-epoxide (Fig. 1). As suggested by these

authors, the metabolism of strychnine appeared to be associated with detoxification, with the 2-hydroxystrychnine, 16-hydroxystrychnine and strychnine *N*-oxide products of relatively low toxicity. Research reported by Nguyen-dang and Bisset (1968) using a strychnine-treated fungal culture (*Helicostylum piriforme*), indicated the presence of the *N*-oxide product within 2 days, followed by the appearance of 16-hydroxystrychnine at the 7-day sampling period (Fig. 2). Bucherer (1965), using a bacterial culture containing no auxiliary source of nitrogen, indicated the presence of mainly low molecular weight degradation products of strychnine (Fig. 2). Niemer and Bucherer (1962) also studied the metabolism of strychnine by *Arthrobacter*, but added an inorganic nitrogen source to the strychnine-containing incubation medium; they demonstrated the presence of C_{16} hanssenic acid, which resulted from the indole ring splitting from the remainder of the strychnine molecule (Fig. 2).

This study was designed to evaluate loss of strychnine (non-radiolabeled) from soil by determining the residual concentrations of the parent compound in soil during a 2-month incubation period, as an indirect measurement of biodegradation, and to attempt to detect major extractable products in the two different soils using a two-step extraction procedure and HPLC/u.v. analysis.

The various metabolites of strychnine that were identified in previous metabolism research and presented in Figs 1 and 2, are discussed in this paper in reference to a possible mechanism by which strychnine may degrade in soils.

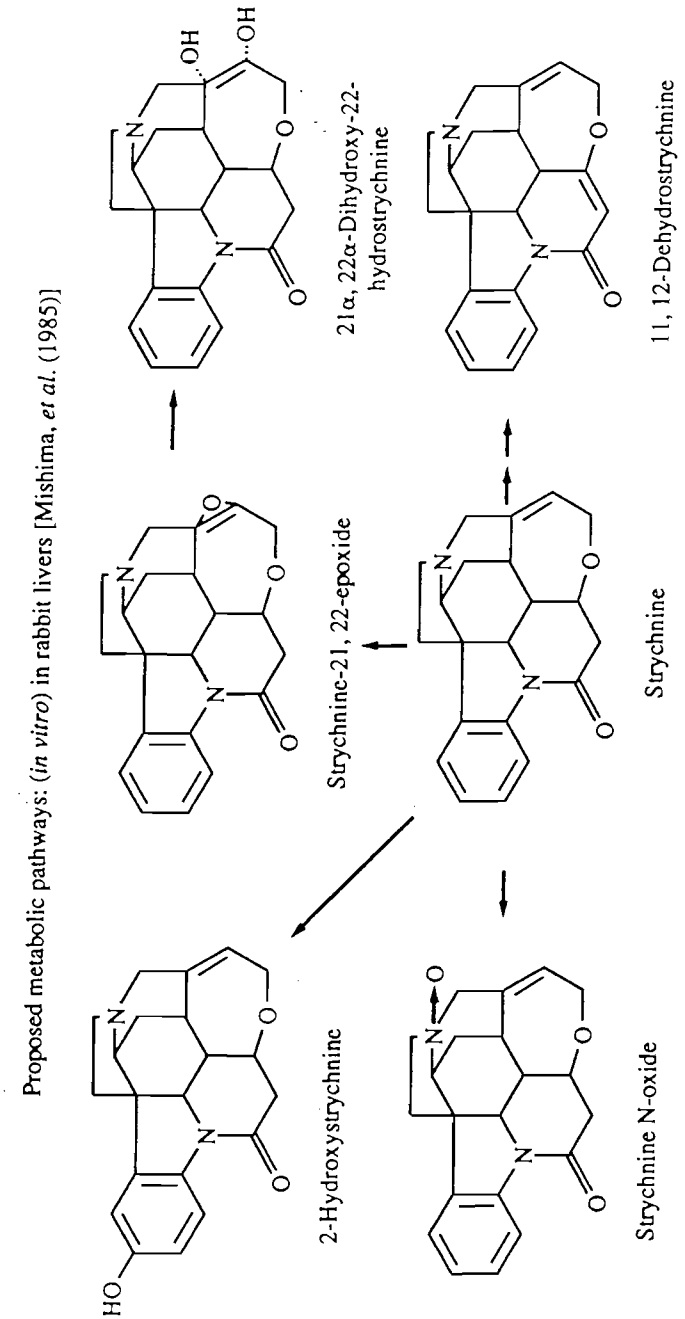
METHODS

Strychnine alkaloid

The strychnine alkaloid (free base, reagent grade, CAS 57-24-9) used in the study for treatment of the soil samples was purchased from the Sigma Chemical Company, St Louis, MO. The chemical was assayed using HPLC with u.v. detection at 254 nm and was determined to have a mean purity value of 101·5% (coefficient of variation 0·87%, $n = 5$).

Soil selection and preparation

The sandy loam and sandy clay loam soils (Ascalon series) were collected in Larimer County, Colorado, during September and October 1993. Collection of the two soils involved removal of approximately the upper 10 cm of surface soil. The chemical and physical properties of the two


Proposed metabolic pathways: (*in vitro*) in rabbit livers [Mishima, *et al.* (1985)]
21α, 22α-Dihydroxy-22-hydrostrychnine
11, 12-Dehydrostrychnine
Strychnine-21, 22-epoxide
Strychnine
2-Hydroxystrychnine
Strychnine N-oxide

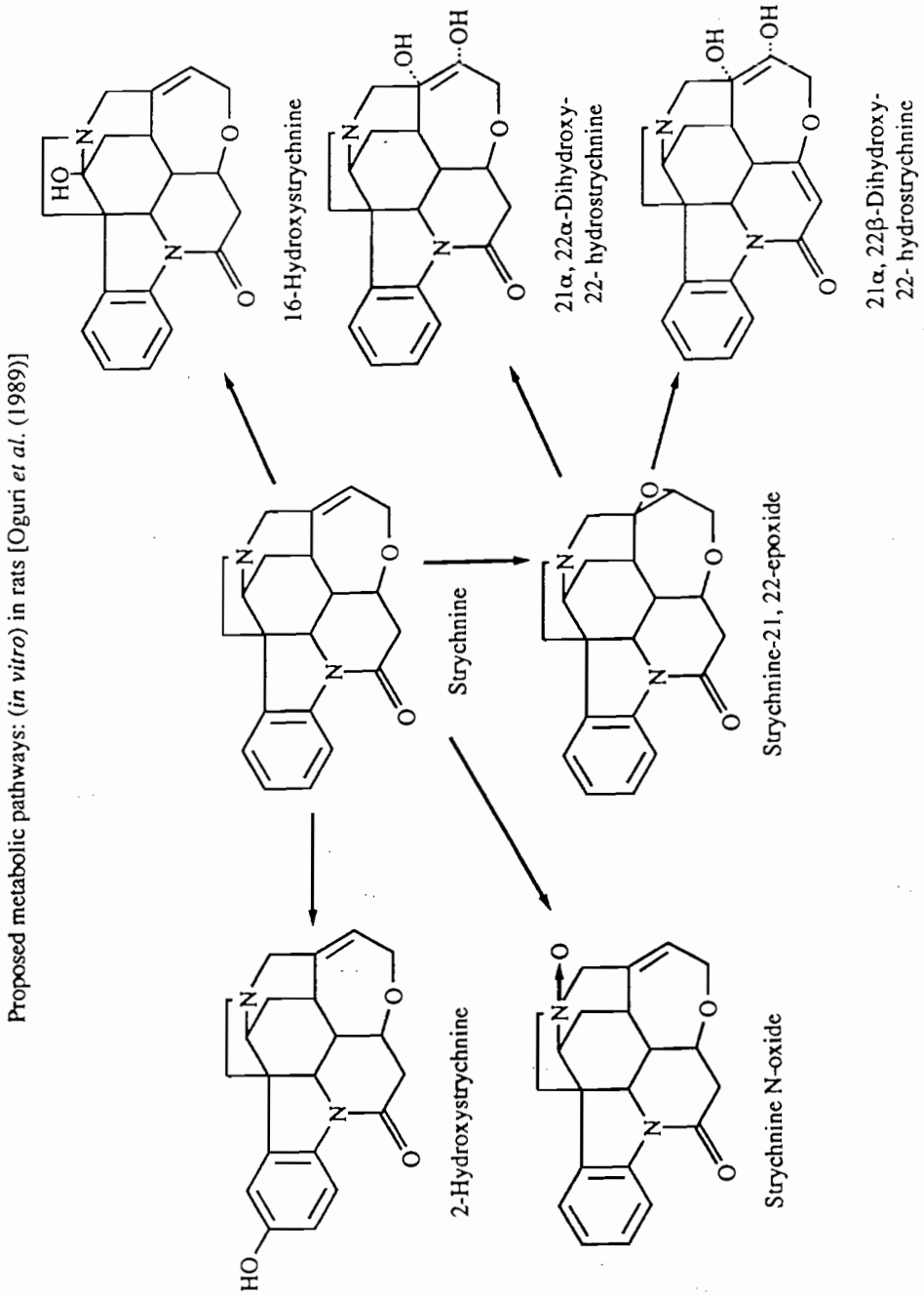


Fig. 1. Proposed metabolic pathways of strychnine in rabbits and rats.

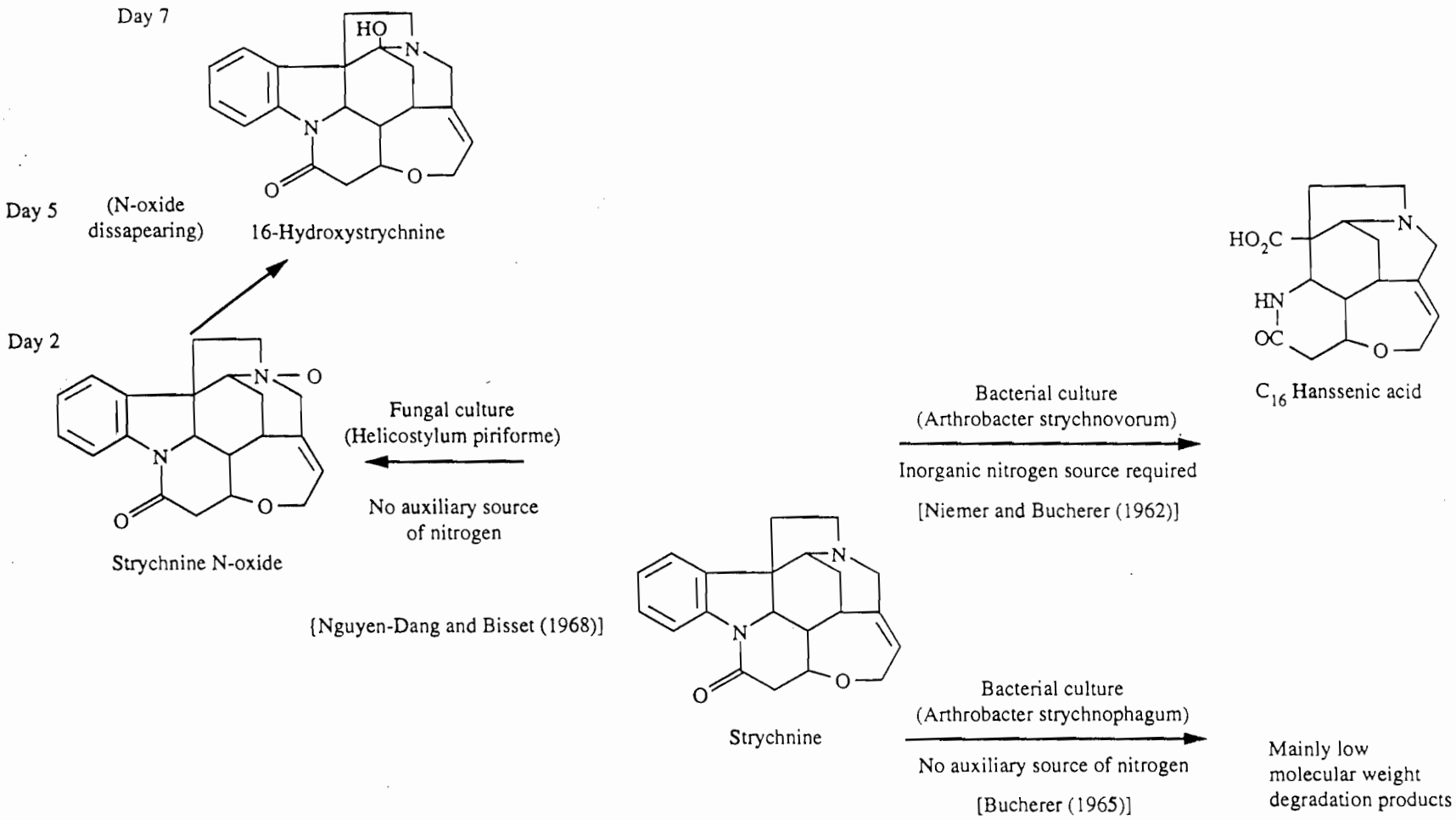


Fig. 2. Proposed degradation pathways of strychnine in fungal and bacterial cultures.

different soils are presented in Table 1. These soils were considered representative of soils in the western United States where strychnine-treated baits will be used as a minor-use pesticide for control of agricultural damage by various rodent species. The soils were dried and then sieved, after first removing plant fragments, collecting the fraction that passed through a 2-mm sieve. The soil was thoroughly mixed, placed in polypropylene bags and stored under refrigeration at about 3–4°C until used (2–4 weeks), to minimize changes in the microbial populations.

Soil incubation apparatus and procedures

The study was conducted using Kimax 250-ml Erlenmeyer flasks with solid glass stoppers that were modified to contain full-length slots (about 2 mm width × 2 mm depth) to assure that aerobic conditions were maintained during the incubation period.

Strychnine alkaloid was dissolved in methanol at a concentration of about 487 μg/ml, as verified using HPLC/u.v. Twenty-gram quantities of the dry test soils were weighed into 250-ml Erlenmeyer flasks. The soil samples were uniformly surface-treated with 1 ml (about 487 μg) of the strychnine solution. After evaporation of the methanol solvent from the soils for about 16–17 h under a fume hood, an additional 30 g of each respective soil type were weighed into each Erlenmeyer flask; the soil was then mixed thoroughly by stirring and rotating the flask. This procedure resulted in about 10 ppm of strychnine, based upon uniform distribution of the chemical within the soil. Control soil samples were treated with 1 ml of methanol.

After soil treatment, deionized water (pH 6·6) was added dropwise to each of the soil samples, until a final moisture content approximating 75% of field capacity was obtained. After a thorough mixing of the soil by stirring, the flasks, containing slotted glass stoppers, were placed in an environmental chamber and maintained under dark conditions at a temperature of 25° ± 1°C during the 2-month incubation period.

The soil flasks were removed from the chamber every 6 or 7 days and deionized water was added to each sample to replenish (by weight) that quantity which had evaporated, and thus maintain the proper soil moisture content. The mean moisture evaporation rate during the 2-month incubation period for both the sandy loam and sandy clay loam soils was about 0·01 g per day.

Soil sampling periods

Eight sampling periods were chosen, based upon results of previous biodegradation research (Starr *et al.*, 1992): time of treatment ($t = 0$) and 7, 14,

TABLE 1
Chemical and Physical Properties of the Ascalon Sandy Loam and Sandy Clay Loam Soils

Texture	*%*				*pH (paste)*	*CEC (meq/100 g)*	*H_2O at $\frac{1}{3}$ bar (%)*
	Sand	*Silt*	*Clay*	*Organic matter*			
Sandy loam	73	10	17	1·7	7·6	11·4	10·9
Sandy clay loam	60	13	27	2·3	7·9	18·8	16·9

21, 28, 35, 42 and 56 days following treatment. At each time interval, three treated samples and a control were selected according to a prearranged random sampling schedule for strychnine extraction and analysis.

Soil microbial population counts

In order to verify the microbial viability of the two test soils, and to evaluate possible effects of 10 ppm strychnine upon microbial populations, soil microbial growth was monitored 7 and 56 days following treatment. At each sampling period, triplicate treated and control soil samples were analyzed.

Five replicate petri plates containing solidified nutrient agar were inoculated with 0·1 ml-aliquots of an appropriate dilution of each soil sample. The plates were then incubated at 28°C for 5 and 10 days for the counts of fungal/bacterial and actinomycete colonies, respectively, in accordance with the methods of Wollum (1982).

Strychnine extraction and analysis

The use of n-butyl chloride as a soil extractant has been demonstrated in our laboratory to be effective in removing strychnine residues from the soil matrix (Hurlbut *et al.*, 1992; Starr *et al.*, 1992; Timm *et al.*, 1993). However, since possible soil degradates may be more polar than the parent strychnine, a two-step extraction procedure was developed.

Methanol extraction

The incubated soil samples (50 g), each contained in a 250-ml Erlenmeyer flask, were first extracted with 200 ml of HPLC-grade methanol. The flask, after being sealed with a solid glass stopper, was placed on a mechanical shaker for 15 min; after the particulate matter had settled, the methanol solution was decanted into a stainless steel parabolic filtering bell. Using vacuum, the liquid was filtered through a 0·2 μm Teflon® membrane into a 250-ml Erlenmeyer flask. The solution was then evaporated using a hot water bath (≈68°C) under nitrogen to a volume less than 25 ml. The residual solution was then transferred to a 25-ml volumetric flask and diluted to volume with methanol. An aliquot of this solution was filtered through a 0·45 μm Teflon® filter disk into a glass vial for analysis by HPLC.

n-Butyl chloride extraction

After evaporation of residual methanol from the soil, 15 ml of a 1·5 N NaOH aqueous solution were added to the dry sample. The sample

container was sealed and shaken by hand to distribute the NaOH throughout the soil. *n*-Butyl alcohol (5 ml) was added to the sample to reduce the possibility of emulsion formation during the soil extraction. To extract strychnine and other weakly polar compounds from the soil, 100 ml of n-butyl chloride were added to the sample. The container was sealed and placed on a mechanical shaker for 10 min. After decanting the extractant into a 250-ml Erlenmeyer flask, the solution was evaporated on a hot plate (≈100°C) under nitrogen. The soil sample was then extracted two additional times using n-butyl chloride as described above. After evaporation of the combined extracts to dryness, 2 ml of n-butyl alcohol were added to the flask to dissolve residual material; to this solution, 50 ml of a diluent [5 mM aqueous heptanesulfonic acid solution and aqueous acetonitrile (70%:30%)] were added to the flask. After sealing the flask, the solution was sonicated for 15 min. An aliquot of this solution was withdrawn and filtered through a 0·45 μm nylon filter into a glass vial for strychnine analysis by HPLC.

The strychnine extraction efficiency was about 89% from 50-g sandy loam and sandy clay loam samples that had been spiked with 15 ppm of the compound.

The liquid chromatographic operating parameters, using a Hewlett–Packard 1090 or 1050 HPLC equipped with a diode array detector, were as follows.

Methanol Extracts (Polar Degradates)

Injection volume:	100 μl			
Flow rate:	0·75 ml/min			
Run time:	10 min — standard solutions			
	60 min — study samples			
Detector:	u.v. @ 254 nm, 210 nm, 300 nm, 300 nm, cell slit width 8 nm			
Column:	CN, 150 × 4·6 mm i.d., 7μ packing, guard column (C8)			
HPLC:	Hewlett–Packard 1050			
Temperature:	ambient			
Mobile phase:	*Isocratic* — standard solutions			
	60% — 0·05 M KH_2PO_4 buffer			
	40% — acetonitrile			
	Gradient — study samples			
	0·05 KH_2PO_4	60% 7 min	> 85% 5 min	> 60%
	Acetonitrile	40% (10 min)	15% (5 min)	40% (33 min)

n-Butyl Chloride Extracts (Strychnine Residues)

Injection volume:	50 μl
Flow rate:	1·0 ml/min
Run time:	9 min — standard solutions
	25 min — study samples
Detector:	u.v. @ 254 nm
Column:	Keystone C8, 250 × 4·6 mm i.d., 5μ packing, guard column (C8)
HPLC:	Hewlett–Packard 1090
Temperature:	Ambient
Mobile phase:	*Isocratic* — standard solutions
	72% — IPC B7
	28% — acetonitrile
	Gradient — study samples

IPC B7	72% 7 min	>25% 2 min	>72%
Acetonitrile	28%	75%	28%
	(9 min)	(2 min)	(10 min)

Standard solutions of strychnine, prepared in both methanol and 5 mM heptansulfonic acid and acetonitrile (70:30%), were used to generate calibration curves for strychnine quantitation.

A linear relationship was observed between chromatographic peak response and strychnine concentration (0·05–1·01 μg/ml) for standard solutions prepared in methanol. Also, this same relationship was observed for standard solutions of strychnine prepared in 70:30% 5 mM heptanesulfonic acid solution:acetonitrile, over the ranges of 0·01–5·06 μg/ml and 5·01 and 15·2 μg/ml; linearity was determined by inputing the peak response data into a linear regression analysis program (SAS Institute, Cary, NC).

The smallest quantity of strychnine that could be detected and definitely attributed to a strychnine chromatographic response was determined to be 0·9 μg of the alkaloid, as applied to 50 g of either the sandy loam or sandy clay loam soil, and extracted using the n-butyl chloride solvent system.

RESULTS

Biological viability of the two test soils

Both test soils were biologically active, as determined by microbial plate counts (Tables 2 and 3). These population counts were made on triplicate soil samples that had been reactivated for 7 days at a moisture content of about 75% of field capacity.

TABLE 2

Microbial Abundance in a Moist Sandy Loam Soil[a] Following Treatment with 10 ppm Strychnine

Days after treatment	*Number of colony-forming-units per gram dry soil (mean ± 1 SD, n = 5)*					
	Bacteria (× 10^6)		*Actinomycetes (× 10^5)*		*Fungi (× 10^3)*	
	Control	*Treated*	*Control*	*Treated*	*Control*	*Treated*
7	16·8 ± 2·75	15·6 ± 2·83	20·0 ± 2·42	18·6 ± 2·85	14·8 ± 1·3	19·2 ± 2·93
	15·7 ± 2·84	16·9 ± 2·24	18·6 ± 2·86	21·5 ± 2·54	16·7 ± 2·74	16·7 ± 2·52
	17·0 ± 3·10	16·3 ± 2·44	20·0 ± 4·72	20·0 ± 2·37	17·0 ± 3·1	16·9 ± 2·70
56	16·8 ± 2·57	16·0 ± 1·73	88·0 ± 15·9	86·1 ± 13·7	12·8 ± 1·87	14·1 ± 2·05
	15·4 ± 2·89	14·8 ± 1·36	89·5 ± 18·9	81·3 ± 13·6	9·99 ± 2·03	14·4 ± 2·59
	13·6 ± 2·23	15·9 ± 3·09	98·6 ± 12·0	73·4 ± 12·8	10·9 ± 2·41	12·4 ± 1·87

[a] Soil previously cropped in beams.

TABLE 3

Microbial Abundance in a Moist Sandy Clay Loam Soil[a] Following Treatment with 10 ppm Strychnine

Days after treatment	*Number of colony-forming-units per gram dry soil (mean ± 1 SD, n = 5)*					
	Bacteria (× 10^6)		*Actinomycetes (× 10^5)*		*Fungi (× 10^3)*	
	Control	*Treated*	*Control*	*Treated*	*Control*	*Treated*
7	2·46 ± 0·94	4·26 ± 1·46	17·9 ± 2·86	29·3 ± 4·44	5·82 ± 0·94	5·38 ± 0·50
	6·38 ± 2·12	3·58 ± 1·23	19·8 ± 3·01	28·7 ± 3·03	4·84 ± 1·48	4·93 ± 0·61
	5·06 ± 1·67	2·89 ± 0·99	19·1 ± 1·84	28·0 ± 4·33	4·62 ± 1·43	5·99 ± 0·61
56	23·0 ± 2·12	24·5 ± 1·81	216 ± 26·6	207 ± 26·3	8·39 ± 1·26	10·8 ± 0·49
	21·1 ± 1·80	24·4 ± 2·84	213 ± 28·6	204 ± 31·7	8·34 ± 1·25	11·9 ± 1·81
	19·8 ± 2·33	24·7 ± 3·97	207 ± 23·8	209 ± 23·3	10·1 ± 1·20	13·2 ± 2·06

[a]Soil previously cropped in corn.

Recovery of strychnine from soil samples incubated for a 2-month period

The mean recovery of strychnine from triplicate samples at each sampling point is presented in Table 4; as illustrated, strychnine applied at a concentration of 10 ppm to freshly collected and biologically active sandy loam and sandy clay loam soils, was found to dissipate (degrade) over a 2-month period, when incubated at a temperature of 25°C and a soil moisture content of 75% of field capacity.

Strychnine degradation kinetics

Degradation of pesticides and other organic chemicals in soil is dependent upon the chemical concentration (C) and the microbial population (X).

If the microbial population is assumed to remain constant over the entire study incubation period, a pseudo first-order rate expression can be developed as illustrated in equation (1), where k is the pseudo first-order rate constant:

$$-\mathrm{d}C/\mathrm{d}t = k\,[C]\,. \tag{1}$$

Integration of equation (1) yields equation (2), where C_0 and C_t are the concentration of test chemical at time 0 and time t:

$$\ln C_t/C_0 = -kt\,. \tag{2}$$

Thus, a plot of $\ln C_t/C_0$ vs t will yield a straight line with a slope of k, which is equal to the degradation rate constant. Once the slope is determined, the half-life ($t_{1/2}$) can be calculated from equation (3):

$$t_{1/2} = \ln\frac{2}{k}. \tag{3}$$

When the strychnine recovery data for both soil types were plotted in this manner (i.e. $\ln C_t/C_0$ vs incubation time), sigmoidal-like recovery curves were obtained, with the strychnine loss from the treated soils occurring in three distinct phases (Table 4, Figs 3 and 4). A discussion of the three phases is presented below.

Lag phase

During the initial lag phase, about 93% of the strychnine application was recovered from the sandy clay loam soil at the end of this 21-day period (Table 4). Although a lag period was observed with the treated sandy loam soil before a rapid loss of the alkaloid occurred (Table 4), the lag period was not as apparent, with about 71% of the strychnine application recovered at day 21 (Figs 5 and 6).

TABLE 4

Recovery of Strychnine from Ascalon Sandy Loam and Sandy Clay Loam Soils Treated with About 10 ppm[a] of the Alkaloid and Sampled at Various Intervals over a 2-month Incubation Period

Period of incubation (days)	*Strychnine recovery*			
	Sandy loam soil		*Sandy clay loam soil*	
	Mean concentration (μg ± 1 SD)	*% of applied*[b]	*Mean concentration (μg ± 1 SD)*	*% of applied*[b]
7	410 ± 6	89·1	422 ± 1	98·4
14	389 ± 15	84·6	411 ± 13	95·8
21	326 ± 20	70·9	397 ± 2	92·5
28	116 ± 60	25·2	217 ± 29	50·6
35	25 ± 4	5·4	86 ± 16	20·1
42	14 ± 11	3·0	28 ± 28	6·5
56	11 ± 2	2·4	33 ± 15	7·7

[a] Strychnine treatment rate about 487 μg per 50 g soil.

[b] Values based upon the mean recovery (observed) at time of treatment of 460 and 429 μg strychnine from replicate samples of sandy loam and sandy clay loam soil, respectively.

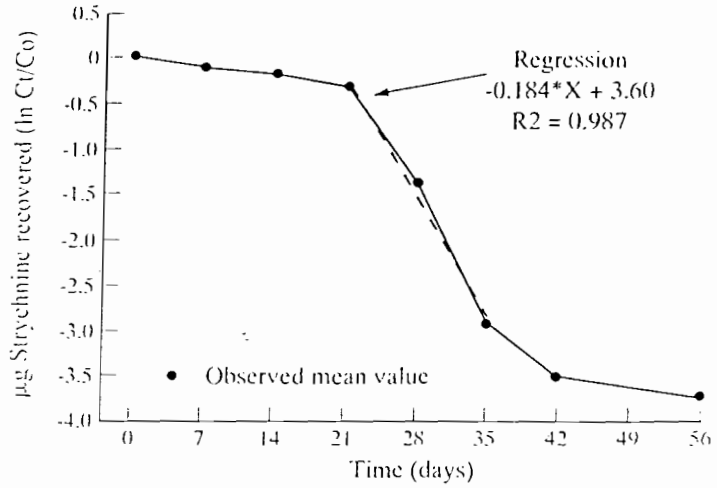


Fig. 3. Natural log plot of strychnine recovery vs. incubation time for a sandy loam soil treated with 10 ppm of the chemical.

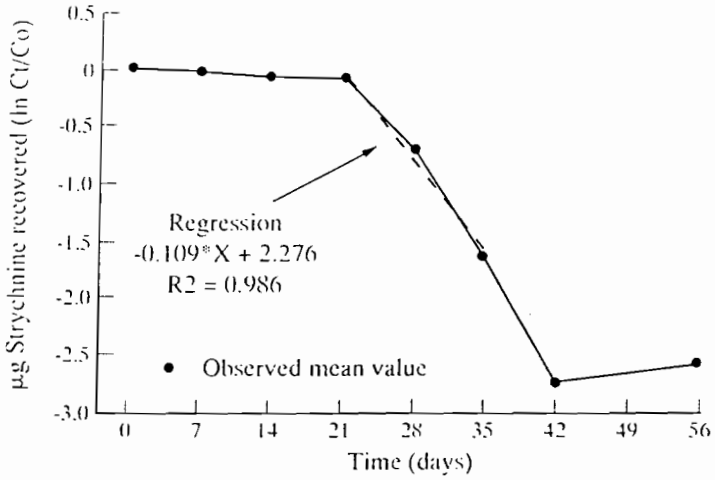


Fig. 4. Natural log plot of strychnine recovery vs. incubation time for a sandy clay loam soil treated with 10 ppm of the chemical.

The lag phase may be the result of a combined microbial adaptation period and soil sorption. Thus, with both soils, which had not received any prior applications of strychnine, the degradation is suggested to have followed kinetics typical of microbial metabolism, in which the slow or lag phase was followed by a rapid or exponential loss of strychnine (Figs 3 and 4). During the initial slow phase, an increase in the microbial populations to a suitable size may have been required before substantial breakdown of strychnine could take place. The microbial abundance data (Tables 2 and 3) illustrate differences in fungal and bacterial populations of the two soils 7 days following reactivation of the microbes. As illu-

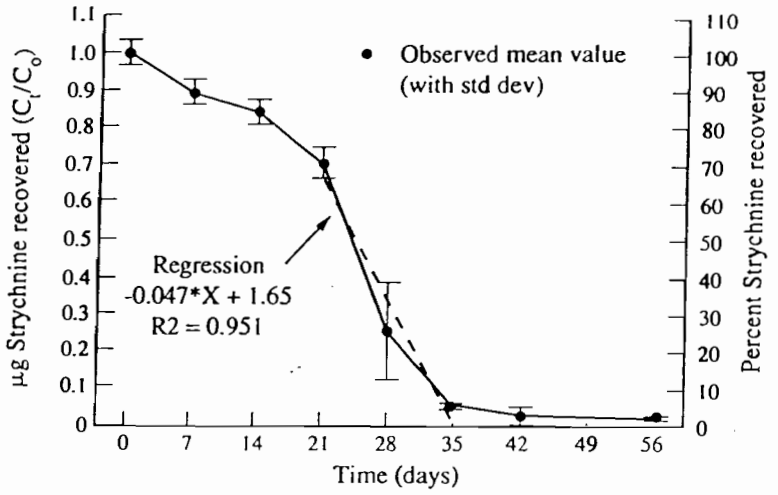


Fig. 5. Strychnine recovery from a sandy loam soil treated with 10 ppm of the chemical and incubated for 2 months.

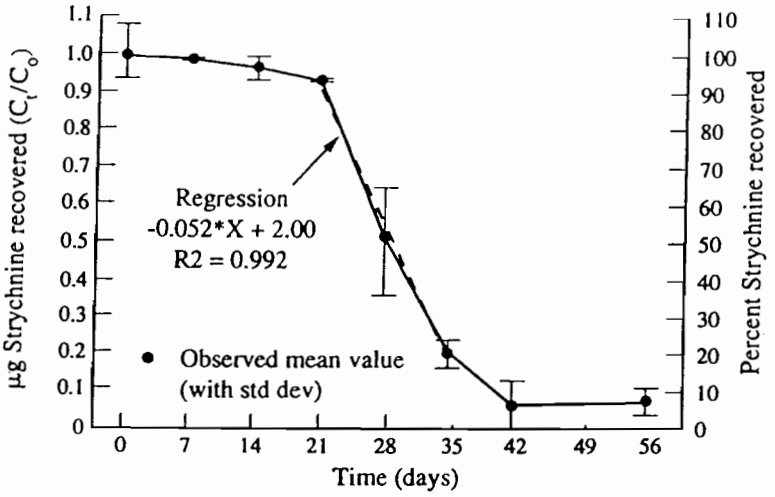


Fig. 6. Strychnine recovery from a sandy clay loam soil treated with 10 ppm of the chemical and incubated for 2 months.

strated in Tables 2 and 3, the sandy loam soil area that had been previously planted with a legume crop (beans) contained a larger initial bacterial population than did the sandy clay loam area that had been cropped in corn. Also, the initial fungal activity in the sandy loam was greater than that of the sandy clay loam soil. Thus, the larger fungal and bacterial populations in the sandy loam soil could have attributed to a less-defined strychnine lag phase in this soil.

Lag phases prior to the degradation of various pesticides have been previously reported to vary from a few days or weeks, depending upon the compound, microorganisms, and soil medium (Parker & Doxtader, 1983;

Starr & Cunningham, 1975; Starr *et al.*, 1992; Burns & Gibson, 1980; Sandmann *et al.*, 1988).

Rapid loss phase

During this period, beginning at about day 21 and extending to about day 35, loss of strychnine from the two different soils occurred in essentially an exponential manner (Figs 3 and 4). At the end of this rapid loss phase (day 35), about 5 and 20% of the strychnine remained in the sandy loam and sandy clay loam soils, respectively (Table 4).

Soil binding phase

The apparent decline in the rate of strychnine loss (days 35–56) from both soils (Figs 5 and 6), is suggested to be due to soil binding, whereby residual chemical is bound within the soil colloidal matrix. At day 56 of the study, between 2 and 8% of the initial strychnine application was extractable from the two test soils (Table 4).

For a complex system such as soil, it is reasonable to expect that a single rate law would not apply to the degradation of a large alkaloid molecule such as strychnine, since the reaction rate would undoubtedly be modified and complicated by the ongoing biological processes, combined with the sorption and diffusion processes (Hamaker, 1972).

Although the kinetics of strychnine loss from the Ascalon sandy loam and sandy clay loam soils cannot be expressed using a single pseudo first-order rate constant, the degradation rate can be described in terms of time observed experimentally for 50 and 90% dissipation of the parent chemical. Dissipation times (DT) are provided in Table 5. As illustrated, DT_{50} values of 24 and 27 days were estimated for strychnine in the sandy loam and sandy clay loam soils, respectively; within periods of 33 and 40 days, the majority (90%) of the strychnine had degraded in the two soils.

TABLE 5
Dissipation Times for 50 and 90% Loss of 10 ppm Strychnine from Two Different Soils Incubated for 2 Months at 25°C and 75% Moisture

Soil type	DT^{a}_{50} *(days)*	DT^{a}_{50} *(days)*
Sandy loam	24	33
Sandy clay loam	27	40

[a]Taken from sigmoidal plots of strychnine recovery vs. incubation time (observed values).

Effects of strychnine upon soil microbial populations

In general, strychnine did not appear to affect microbial populations of either test soil, when applied at a concentration of 10 ppm (Tables 2 and 3). Possible strychnine effects were evaluated in terms of population level changes of bacteria, actinomycetes and fungi in incubating soil samples treated with strychnine as compared with untreated control (methanol only) samples.

Bacteria

There were no apparent effects of strychnine observed upon bacterial populations in either soil 7 or 56 days following treatment.

Actinomycetes

A slight stimulative effect upon the actinomycete population may have occurred in the treated sandy clay loam soil 7 days following treatment; this effect, however, was not observed at the 56-day sampling period. No effects from strychnine were observed in the sandy loam soil actinomycete population at either the 7- or 56-day sampling periods.

Fungi

Strychnine did not appear to affect fungal populations in either the sandy loam or sandy clay loam soils 7 or 56 days following treatment.

Detection of strychnine degradation products

Analysis of methanol extracts of the treated and control sandy loam and sandy clay loam soils using HPLC/u.v. revealed the presence of a secondary peak in treated sample extracts early in the study (day 7), which reached a maximum height at either day 14 (sandy loam) or day 21 (sandy clay loam); the peak then decreased in height with increased incubation time. The peak was not detected in any of the sandy loam methanol extracts after day 21; although the peak height decreased with time in extracts of the sandy clay loam soil, the peak was still detected in one of the sandy clay loam replicate samples at days 42 and 56 (Fig. 7). No attempt was made to isolate and identify the product.

Considering the large size of the strychnine alkaloid molecule, it is surprising that additional peaks, representing a variety of possible non-volatile products, were not detected in soil extracts over time, especially during the rapid strychnine loss period. The fact that other products were not detected could be explained if the products that formed were rapidly and tenaciously sorbed to the soil colloids and not extracted

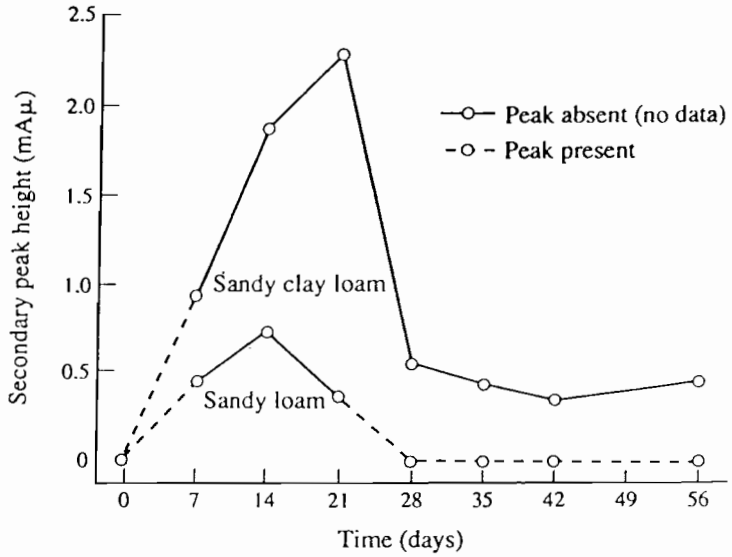


Fig. 7. Secondary peak detected in methanol extracts of two different soils.

with either of the solvent systems, or were rapidly degraded by soil microorganisms.

DISCUSSION

From the indirect biodegradation data presented in this paper, strychnine alkaloid, applied at a concentration of 10 ppm to two different soils, was found to dissipate over a 2-month period, when incubated at a temperature of 25°C and a soil moisture content of 75% of field capacity. The manner by which strychnine loss occurred in this study and in other biodegradation research conducted at this laboratory (Starr *et al.*, 1992), may suggest a random type of enzymatic adaption or mutation, followed by development of a sufficient population of strychnine-degrading organisms. During the initial lag phase, an increase in populations of the appropriate microorganisms may have been required before substantial breakdown of strychnine could take place. Moreover, the larger initial fungal and bacterial populations in the sandy loam soil may have attributed to a less-defined strychnine lag phase in this soil.

Possible mechanism by which strychnine degraded in the two soils

As indicated from the discussion contained in the introduction of this paper, certain degradation products of strychnine have been identified that are common, regardless of the biological system. Thus, it is possible that the breakdown products that formed during the 2-month incubation period in this study, may be the same as certain of the polar products

illustrated in Figs 1 and 2. With this in mind, a discussion of the means by which strychnine may have dissipated (degraded) in the sandy loam and sandy clay loam soils is presented below.

Upon application of strychnine to the dry soils, the chemical was rapidly sorbed to the soil colloids. Following remoistening of the soils to about 75% of field capacity, and during a 21-day lag phase, strychnine sorption/desorption processes were likely ongoing, combined with microbial adaptation — evidence of a breakdown product was suggested to have occurred in both soil types during this period. This adaptative phase may have involved one or more of the following: (1) induction of enzymes specific for degradation of the alkaloid; (2) random mutation by which new metabolic capabilities were produced that allowed breakdown of the chemical to occur; and/or (3) increase in the number of strychnine-degrading organisms. Random microbial mutations of this type would serve to help explain the variability in strychnine recovery results observed between sample replicates in previous soil biodegradation research that we have conducted at this laboratory (Starr *et al.*, 1992).

During the exponential degradation phase (days 21–35), a rapid loss of the chemical occurred, once a sufficient concentration of strychnine desorbed from the soil and became available in the soil solution, and as microbial activity became sufficiently great.

If one assumes that the initial breakdown product(s) of strychnine were similar to those observed in previous metabolism studies, i.e. strychnine *N*-oxide, 16-hydroxy strychnine and/or 2-hydroxy strychnine, plus other possible products, these polar degradates were probably bound rapidly and strongly to the soil colloidal fractions, which resulted in the exponential loss of strychnine from the soil solution during the rapid loss phase (Figs 3 and 4). These bound product residues would be anticipated to be slowly further degraded by microorganisms, and eventually to be completely mineralized to carbon dioxide, together with the organic matter fraction. Prior to further breakdown of the initial degradation products, movement of these bound compounds within either soil would probably be low.

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